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Analysis of pesticide residues in stingless bee honey samples in Karnataka, India

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Abstract
Honey of stingless bee is composed of different chemical compounds along with toxins, pesticide residues and other environmental pollutants. The presence of pesticides residues and other contaminants and several of them are potential carcinogens which cause adverse health effects in man. Published reports on pesticides residues analysis in the honey of stingless bees is poor and hence the present investigation was made using various standard methods. Total 226 pesticides residues were found in the honey samples collected from Mysore, Chamarajanagar and Kodagu districts of Karnataka. Interestingly, all these 226 pesticides residues were found below detection limits. Moreover, among the screened pesticides residues, residues of the herbicides were more (66) and it was followed by residues of fungicides (50), general insecticides (43) and organophosphates (16) found in the honey of stingless bees. Further, residues of the plant growth regulators (Nine), acaricides (Eight), organochlorines (Six), organothiophosphates and pyrethroids (Five each), carbamates and neonicotinoids (Four each), organophosphorus compounds (Two), organofluorine, plant growth retardant, insect growth regulator and antibiotic compounds (One each) were also found in the honey of stingless bees. Surprisingly, all these pesticides residues were found below detection limit in the honey sample and indicated that honey is free from heavy concentration of pesticides residues and safe for human use as per the international standards, further in depth studies are required on animal models.

Keywords: Pesticides residues, stingless bee honey, Karnataka

Introduction
Stingless bee honey has good curative properties against various ailments. Honey possesses good antimicrobial activity with less sugar content as compared to honey derived from *Apis* species. Honey is composed of more than 300 chemical compounds, but mainly carbohydrates (>75%), water (~18%) with minor components comprising of proteins, amino acids, vitamins, antioxidants, minerals, essential oils, sterols, pigments, phospholipids and organic acids (Bogdanov *et al.*, 2008 [13]; Kujawski and Namiesnik 2008). Kujawski and Namiesnik (2008) [33] have recorded diverse range of chemical compounds with toxins, pesticide residues and other environmental pollutants. The presence of pesticide residues and other contaminants in honey have adverse health effects on honeybees and deteriorated the quality of honey and devalue its beneficial properties (Bogdanov *et al.*, 2008) [13]. Typically, pesticide residues in honey occurs when bees in search of nectar/pollen when they visit to flowering plants or agricultural crops which have been treated with various agro-chemicals and/or pesticides to control pests or diseases (Bogdanov, 2006) [13]. Due to pesticides application on crops, it contaminates the soil, air, water, flowers, when honeybees collect nectar/pollen from such flowers it results contaminated honey production (Kujawski and Namiesnik, 2008) [33]. In India, many farmers are growing various types of commercial crops where, huge quantity of pesticides are used to safeguard their agricultural crops from different species of pest's infestation (Basavarajappa, 2011) [8]. Hence, there is a wide scope to contaminate flowers from pesticides. Moreover, unscientific use of toxic pesticides during blooming periods of agricultural and horticultural crops not only kills honeybees but also contaminates hive products such as honey and pollen (Rissato *et al.*, 2007) [48]. The harmful pesticides enter into beehive through nectar or pollen when offered by honeybee species. When once those pesticides introduced into the honeycomb, the honey gets contaminated and becomes unfit for human consumption. Moreover, pesticides contamination most often affects the physico-chemical properties of honey and finally alter the physical and

biochemical properties of honey (Basavarajappa *et al.*, 2011) [8]. Table 1 shows the published reports on the analysis of honey for its pesticides residues across the world. Honey is being used from ancient times, it has attracted the attention of man, especially due to its sweet taste (Bera and Almeida-Muradian, 2007) [3]. Hence, honey is one of the most commonly used viable products in human society and commercially used in industrialized forms (Komatsu *et al.*, 2002) [32]. The health and nutritional benefits of honey are reduced if it is contaminated with toxic chemicals such as residues of pesticides (Blasco *et al.*, 2004) [12] and other environmental contaminants. All pesticides residues are toxic and several of them are potential carcinogens which may cause chromosomal aberrations (McDougal and Safe, 1998) [60]. Pesticides residues are also known to cause changes in the endocrine system (Brander *et al.*, 2016), reproductive system and nervous system in insects including honeybee species.

Several researchers have reported the pesticides residues in honey at varying concentrations (Blasco *et al.*, 2003; Martel *et al.*, 2007; Rissato *et al.*, 2007; Choudhary and Sharma, 2008; Wang *et al.*, 2010; Blasco *et al.*, 2011; Weist *et al.*, 2011; Kujawski and Namiesnik, 2011; Basavarajappa, 2011; Barganska *et al.*, 2013; Raghunandan and Basavarajappa, 2013, Basavarajappa and Raghunandan, 2014 and Almashi and Basavarajappa, 2019) confirming the necessity to monitor the pesticide residues contamination in honey and to assess the potential health risks to ensure the pure quality honey devoid of any pesticides residues. However, published reports on stingless honey analysis for pesticides residues contamination are poor in Karnataka. Further, monitoring pesticides residues at low levels help avoid potential health risks to their end-users. Furthermore, pesticides in freshly collected honey samples can be used as an indicator of pesticides applied on to nearby agricultural fields. The national and international regulations are showing concern about the permissible levels of pesticide residues in the honey samples. The European Union Regulations considered that honey is a natural product, it must be free from chemical residues and other contaminants. Kasiotis *et al.* (2018) [31], Prashanth *et al.* (2022) have analyzed the honey samples using various methods to identify pollutants, pesticides residues etc. Stingless beekeeping is being promoted all over the world as a source of industry for the generation additional income during different seasons to the farmers. Therefore, regular monitoring of pesticide residues in the honey sample in general and stingless the honey in particular is essential to ensure the quality and safety for human use. Published reports on pesticide residues analysis in stingless bee honey is poor. Hence, the present investigation was made to analyze the pesticide residues in stingless bee honey of Mysore, Chamarajanagar and Kodagu districts of Karnataka.

Materials and Methods

Stingless bee honey samples were collected from Mysore, Chamarajanagar and Kodagu districts from randomly chosen Meliponiculturists. Collected honey sample was stored in a sterilized 50 ml polypropylene tubes and stored at temperature 20^{0}C until analysis.

Chemicals and Reagents used: Solvents used for the mobile phase preparation and extraction such as water, Mass-Spectroscopy (MS) grade procured from Bio-solve (P/N, 232141), Methanol, MS grade procured from Bio-solve (P/N, 136841), and Acetonitrile, MS grade procured from Bio-solve (P/N, 012041). Modifier additive for mobile phase preparation, Formic acid, and Liquid-Chromatography/Mass-spectroscopy (LC-MS) grade was procured from Sigma Aldrich (P/N, 5330020050). Sample preparation kits such as Agilent QuEChERS extraction kit procured from Agilent Technologies (P/N, 5982-5650) sand the cleanup kit, Agilent Bond Elute Dispersive SPE 15 ml procured from Agilent Technologies (P/N, 5982 5058). LCMS/MS comprehensive pesticide test mix was procured from Agilent Technologies (P/N, 5190-0551), whereas Gass Chromatograph (GC) multi-residue pesticide kit of various standards was procured from Restek.

Sample Preparation: Honey samples were brought to room temperature and extracted by the modified QuEChERS method. Two gram honey sample was taken in 50 mL Falcon tube. Five ml Millipore water was added to it to reduce the viscosity of honey samples. The sample was vortexed for one minute to get a homogenized mixture of honey and water, after which 10 ml Acetonitrile (ACN) was added. The mixture was then vortexed for one minute and cooled the contents at -20ºC for 30 minutes. Agilent Bond Elute QuEChERS extraction kit contents were added (P/N, 5982-5650) and shaken vigorously for one minute. The contents of Tarson tubes were centrifuged at 6000 rpm for six minutes. Further, six ml of the supernatant was pipetted out to an Agilent Bond Elute Dispersive SPE 15 ml (P/N, 5982-5058), then contents were thoroughly vortexed for one minute by following centrifugation at 10000 rpm for two minutes and pipetted out one ml solution in HPLC vial. Each of the samples was prepared in triplicates as per AOAC Official Method (2007).

Chromato graphic parameters

A6470ALCMS (G6470AA) Triple Quadruple (Agilent Technologies Inc., Santa Clara, USA) operated in ESI Positive Ionization mode coupled with a 1290 Infinity II UHPLC system (Agilent Technologies Inc., Santa Clara, USA) consists of a high-speed binary pump (G7120A) having a maximum pressure limit of 1300 bar, multi sampler (G7167B) and multi-column Thermostat (G7116B) was used for the sample analysis. MRM transitions were set and optimization of the compound-related voltages such as fragment or voltage and the collision energy was performed by automatic optimization tool and by pesticide MRM database available with the instrument. Source parameters were optimized based on the flow rate and the composition ratio of the mobile phase. Mobile Phase a used was 0.1% formic acid and 5 mM Ammonium Formate in water. 0.1% Formic acid and 5 mM Ammonium Formate in Methanol are used as mobile phase B. Injection volume used was 2ul. The analytical column responsible for the separation of non-volatile pesticides was Agilent Zorbax Eclipse Plus (150X2.1mm, 1.8µm, P/N, 959759-902). The column was kept at a constant temperature of 35ºC. Twenty minute long gradient elution was employed to chromatographically separate the 226 pesticides. The initial composition of the mobile phase was 95.5 (Mobile phase A & B) which was constant for 0.5 minutes. From 0.5 minutes to 3.5 minutes, the mobile phase B composition gradually increased to 50%. At 17.0 minutes, the % B becomes 95%. This ratio was maintained for 20 minutes. A post run of two minutes was also included in the time program for column washing and the stabilization of the column with the initial mobile phase conditions to make it ready for the next injection. Thirty second long washing of the injection needle and the needle seat was also employed using Methanol and water (60, 40; V/V) as wash solvent to minimize the carryover.

MS parameters: ESI positive ionization mode was used to acquire the samples and the standards. A retention time-based MRM method was developed which provide more cycle time for each of the analyte resulting in improved data quality in terms of number data points. Also, standards and samples were acquired in spectral acquisition mode to enable library spectrum matching. Nebulizer pressure was kept at 40 pSi. The heated gas temperature was 200ºC with a flow of 8L/min. Thermal confinement of the expanded spray is obtained with the help of Sheath gas of temperature 325ºC has a flow rate of 11L/min. The capillary voltage was kept at 3500V and the nozzle voltage for the optimized method was 500V. Fragment or Voltages and Collision energies of individual pesticides were obtained from the pesticide MRM database. Wherever, database data was unavailable, an automatic optimizer tool was used to do optimization of voltages. The resolution setting was kept as a unit resolution for both quadruple one and three mass analyzers where the mass spectral peak width is maintained between 0.6 - 0.8Da. Before data acquisition, the status of the MS instrument was verified by the check tune option. Electro Spray Ionization (ESI) low tuning mix provided by Agilent Technologies was introduced by the calibrant delivery system (CDS) Spectral peak width and intensity are measured during this process and warn the user in case it is not meeting the above criteria (Prashanth *et al.*, 2022, SANTE, 2019).

GC-MS/MS method conditions

Chromatographic parameters: For GC-MS/MS analysis, Agilent 7010 pesticide analyzer coupled to GC 7890B ((Agilent Technologies Inc, Santa Clara, USA) was used. Agilent J and WHP 5ms UI 15m × 0.25mm × 0.25µm (P/N, 19091S 431 UI) GC column was used for the separation of analytes. From the inlet, two Agilent J and W DB-5ms Ultra Inert columns (15 m×0.25mm, 0.25µm, p/n19091S-431UI) were coupled to each other through a purged ultimate union (PUU) for the use of mid-column/post-run back flushing. The carrier gas used for GC was Helium with a hot and cold split less injection. Collision and quenching gases were Nitrogen. Purged Ultimate Union was used for back flush. 1ul was injected to analyze the samples. The total run time per sample was 40 minutes. The retention time locking option using chlorpyrifos-methyl was utilized to maintain the retention time of various analytes. From the inlet, two Agilent J and W DB-5ms Ultra Inert columns (15 m × 0.25 mm, 0.25 µm, p/n 19091S-431 UI) were coupled to each other through a purged ultimate union (PUU) for the use of mid-column/post-run back flushing. 2.4.2.2. MS parameters. The GC was configured with a Multimode Inlet (MMI) equipped with a 4 mm ultra-inert, split less, single taper, glass wool liner (p/n 5190-2293). Electron Impact (EI) ionization source with 70ev was used for analysis. The source and the transfer lines were kept at 280oC. Time scheduled MRM (dMRM) method was used for acquisition to get the optimized cycle time for the collection of the maximum number of data points across the chromatographic peaks. EM gain used was 10 with MS1 and MS2 resolutions were kept wide. Both the quadruple analyzers were kept at heated conditions of 150oC so that the fluctuations in outside temperature were not affecting the performance in mass accuracy of the system. For both LC-MS/MS and GCMS/MS at least two MRM transitions consisting of one parent ion and two fragment ions are selected per analyte to satisfy the criteria of four identification points for confirmation of a compound in a sample as per the SANTE guidelines. The MRM transition having more intensity, Quantifier ion, and the second high intense MRM transition Qualifier ion were selected from the production spectra of the pesticides. MRM ratios (Qualifier to Quantifier ratio) were compared between matrix-matched standard and sample of each analyte for confirming any positive sample (Prashanth *et al.*, 2022, SANTE, 2019).

Results

Table 2 shows the 226 pesticides residues were screened for their presence in the stingless bee honey sample collected from Mysore, Chamarajanagar and Kodagu districts of Karnataka. Interestingly, all these 226 pesticides residues were found below detection limit in the stingless bee honey sample collected from this part of Karnataka state (Table 2). Among the screened pesticides residues, residues of the herbicides were more (66) and it was followed by residues of fungicides (50), general insecticides (43) and organophosphates (16) found in stingless bee honey (Table 2). Moreover, residues of the plant growth regulators (Nine), acaricides (Eight), organochlorines (Six), organothiophosphates and pyrethroids (Five each), carbamates and neonicotinoids (Four each), organophosphorus compounds (Two), organofluorine, plant growth retardant, insect growth regulator and antibiotic compounds (One each) were found in stingless bee honey. Surprisingly, all these pesticides residues were found below detection limit in the honey sample collected from Mysore, Chamarajanagar and Kodagu districts of Karnataka (Table2). Thus, stingless bee honey of Mysore, Chamarajanagar and Kodagu districts is free from heavy concentration of pesticides residues, below detection limits and the honey is safe for human use as per the international standards, further in depth studies are required on animal models.

Discussion

Honey is one of the most vital natural products, it must be free from chemical residues and other contaminants. It is known for its good curative properties against various diseases in human beings since prehistoric times (Rossi *et al.*, 1999; Komatsu *et al.*, 2002 [32]; Bera and Almeida-Muradian, 2007) [3, 11]. However, in recent years, it is difficult to obtain pure honey, as it is contaminated with diverse range of chemical compounds, toxins, pesticides residues and various environmental pollutants (Kujawski and Namiesnik, 2008) [33]. During the present investigations, around 226 pesticides residues were found in the stingless bee honey samples collected from Mysore, Chamarajanagar and Kodagu districts of Karnataka. Interestingly, all these 226 pesticides residues were found below detection limits in stingless bee honey samples. Of all the pesticides residues, the residues of herbicides were more predominant (66), followed by residues of fungicides (50), general insecticides

(43) and organophosphates (16) found below detection limits in stingless bee honey. Several researchers have reported the pesticides residues in honey at varying concentrations (Blasco *et al.*, 2003; Martel *et al.*, 2007; Rissato *et al.*, 2007; Choudhary and Sharma, 2008; Wang *et al.*, 2010; Blasco *et al.*, 2011; Weist *et al.*, 2011; Kujawski and Namiesnik, 2011; Basavarajappa, 2011; Barganska *et al.*, 2013; Raghunandan and Basavarajappa, 2013, Basavarajappa and Raghunandan, 2014 and Almashi and Basavarajappa, 2019) at different parts of the world and confirming the necessity to monitor the pesticides residues contamination in honey and to assess the potential health risks to ensure the pure quality honey which should be devoid of any pesticide residues. Surprisingly, local market honey samples are contaminated with different types of pesticide residues in Haryana (Rathi *et al.*, 1997), Portugal and Spain (Blasco *et al.*, 2003) [12], Brazil (Rissato *et al.*, 2007) [48] Egypt (Barakat *et al.*, 2007) [6], Saudi Arabia (Ali *et al.*, 2012) Spain (Fernandez *et al.*, 2002) [25], Switzerland (Bogdanov, 2006) [13] and in Colombia (Rodríguez *et al.*, 2014). Further, *Apis mellifera* honey contains residues of organophosphorus insecticides in Canada (Al-Naggar, 2015) [1], Africa (Irungu *et al.*, 2016) [29], Europe and in Ghana also (Martel, 2018 [36]; Darko *et al.*, 2017) [21]. All these published reports clearly suggested that honey is contaminated with varying concentrations of different pesticides residues.

Further, during the present investigation, residues of plant growth regulators (Nine), acaricides (Eight), organochlorines (Six), organothiophosphates and pyrethroids (Five each), carbamates and neonicotinoids (Four each), organophosphorus (Two), organofluorine, plant growth retardant, insect growth regulator and antibiotic compounds (One each) were found in stingless bee honey. Surprisingly, all these pesticides residues were found below detection limit in the honey sample collected from Mysore, Chamarajanagar and Kodagu districts of Karnataka. Thus, stingless bee honey of Mysore, Chamarajanagar and Kodagu districts is free from heavy concentration of pesticides residues and which are below the detection limits and it requires further in depth investigations to check its quality for human use. Similarly, Codling *et al.* (2016) [20] have recorded the neonicotinoid insecticides in honey, pollen and honey bees in Canada. Organophosphorus insecticides were detected in *Apis mellifera* honey samples in Canada (Al-Naggar, 2015) [1]. Organochlorine residues were also detected in bees and hive products in Karnataka (Singh *et al.*, 2015). Moreover, organochlorine pesticides were detected in honey and pollen samples from managed colonies of *Apis mellifera* in Mexico (Ruiz-Toledo *et al.*, 2018). General pesticide residues were detected in hive products of *Apis cerana indica* at Tamil Nadu (Yogapriya *et al.*, 2023). Neonicotinoid insecticides were identified in honey, pollen and honey bees in Canada (Codling *et al.*, 2016) [20]. However, these reports were collected from *Apis* species honey alone. But, very few published reports are available on the contamination of stingless bee honey due to pesticide residues. Pinheiro *et al.* (2020) and Corolina *et al.* (2020) have reported the pesticide residues in stingless bee, *Melipona* species honey in Brazil. During the present investigation, 226 pesticides residues which belong to herbicides, fungicides, general insecticides, organophosphates, plant growth regulators, acaricides, organochlorines, organothiophosphates, pyrethroids, carbamates, neonicotinoids, organophosphorus compounds, organofluorines, plant growth retardants, insect growth regulator and antibiotic compounds were detected and surprisingly, these pesticides residues/chemical compounds concentration was below detection limits in the stingless bee honey samples.

Pesticide residues in honey have adverse health effects on bees and deteriorate the quality of honey and devalue the honey beneficial properties (Bogdanov *et al.*, 2008) [13]. Moreover, pesticides residues contamination affects the physico-chemical properties of honey and finally alters its physical and biochemical properties of honey (Basavarajappa *et al.*, 2011) [8] thereby alters the nutritional benefits of honey (Blasco *et al.*, 2004) [12] to mankind. Further, many pesticides residues are toxic in nature and many of them are potential carcinogens which may cause chromosomal aberrations, cause changes in the endocrine system (Brander *et al.*, 2016), reproductive system and the nervous system Thus, unscientific excessive use of pesticides residues during blooming periods of agricultural and horticultural crops, weeds in the natural ecosystem not only contaminates hive products such as honey and pollen (Rissato *et al.*, 2007) [48], but also kills honeybees. Thus, monitoring pesticides residues at low levels help avoid potential health risks to their end users. Since, honey is one of the international commodities, used as one of the food items by people across the world. Hence, contamination free honey without any pesticides residues is vital for the health point of end users. Therefore, pesticides residues in honey samples should be regularly monitored to maintain quality in the honey (Deka *et al.*, 2004) [22] as per international standard (Codex, 2000) [19]. The national and international regulations adopted at different countries including India are showing concern about the permissible levels of pesticides residues in the honey samples. Because pesticides residues impact the honeybee colony health (Chauzat *et al.*, 2009) [17] and honeybees in turn on humans (Jivan *et al.*, 2013) [30]. Contaminated honey with pesticides residues have potential risk to consumers (Eissa *et al.*, 2014) [24]. Therefore, pesticide residues in honey samples should be regularly monitored to maintain quality in the honey (Deka *et al.*, 2004) [22] for human use. Our observations are similar to the observations of Rathi *et al.* (1997), Fernandez *et al.* (2002) [25], Blasco *et al.* (2003) [12], Bogdanov (2006) [13], Rissato *et al.* (2007) [48], Barakat *et al.* (2007) [6], Ali *et al.* (2012), Rodríguez *et al.* (2014), Al-Naggar (2015) [1], Irungu *et al.* (2016) [29], Codling *et al.* (2016) [20], Darko *et al.* (2017) [21], Martel (2018) [36], Ruiz-Toledo *et al.* (2018), Kasiotis *et al.* (2021) [31] and Mukiibi *et al.* (2021), Oymen *et al.* (2022), Xiao *et al.* (2022), Murcia-Morales *et al.* (2022) and Yogapriya *et al.* (2023).

Summary

Total 226 pesticides restudies were detected in the stingless bee honey samples and all these pesticides residues were found below detection limits. Among the screened pesticides residues, the residues of herbicides were more (66) and it was followed by the residues of fungicides (50), general insecticides (43) and organophosphates (16). Moreover, residues of plant growth regulators (Nine), acaricides (Eight), organochlorines (Six), organothiophosphates and pyrethroids (Five each), carbamates and neonicotinoids (Four each), organophosphorus compounds (Two), organofluorine, plant growth retardant, insect growth regulator and antibiotic compounds (One each) were found in stingless bee honey below detection limits.

Table 1. Published reports on pesticide residues analysis in honey around the world

Sl. No.	Researched on	Place	Source
1.	Multiresidue analysis of market honey samples for pesticide contamination	Haryana, India	Rathi *et al.* (1997)
2.	Pesticide residue determination in bee products	Spain	Fernandez *et al.* (2002) [25]
3.	Fast and easy multiresidue method employing acetonitrile extraction/partitioning and 'dispersive solid phase extraction' for the determination pesticide residues	Germany	Anastassiades *et al.* (2003) [5]
4.	Assessment of pesticide residues in honey samples	Portugal and Spain	Blasco *et al.* (2003) [12]
5.	Monitoring of pesticide residues in honey samples of Jorhat district, Assam	India	Deka *et al.* (2004) [22]
6.	Contaminants of bee products	Switzerland	Bogdanov (2006) [13]
7.	Simple and rapid method of analysis for determination of pesticide residues in honey	Egypt	Barakat *et al.* (2007) [6]
9.	Development and validation of a multi-residue method for pesticide determination in honey	Belgium	Pirard *et al.* (2007)
10.	Multiresidue determination of pesticides in honey samples by GC-MS	Brazil	Rissato *et al.* (2007) [48]
11.	Pesticide residues in honey samples	Himachal Pradesh, India	Choudhary and Sharma (2008) [18]
12.	Influence of pesticide residues on honeybee colony health	France	Chauzat *et al.* (2009) [17]
13.	Determination of pesticide residues in honey samples	Delhi, India	Mukherjee *et al.* (2009)
14.	Multi-residues analysis of chemical pesticides in imported and locally produced honey	Saudi Arabia	Ali *et al.* (2012)
15.	Impact of pesticides on honeybees and on humans	Romania	Jivan *et al.* (2013) [30]
16.	Analysis of multifloral honey of *Apis dorsata* for pesticide residues	India	Raghunandan *et al.* (2013)
17.	Determination of pesticide residues in honeybees using modified QUEChERS sample work-up and LC-TMS	Poland	Barganska *et al.* (2014) [7]
18.	Determining pesticide residues in honey and their potential risk to consumers	Egypt	Eissa *et al.* (2014) [24]
19.	Multiresidue determination of pesticide residues in honey by modified QuEChERS method and GC with electron capture detection	Brazil	Orso (2014)
20.	Evaluation of pesticide residues in honey	Colombia	Rodríguez *et al.* (2014)
21.	Analysis of multifloral honey of *Apis dorsata* for physical, biochemical and pesticide residues	Karnataka, India	Basavarajappa *et al.* (2014) [8]
22.	Exposure of *Apis mellifera* to organophosphorus insecticides	Canada	Al-Naggar (2015) [1]
23.	Pesticides applies to crops and honeybee toxicity	USA	Egelie (2015) [23]
24.	Organochlorine residues in bees and hive products	Karnataka, India	Singh *et al.* (2015)
25.	Determination of pesticide residues in honey	Africa	Irungu *et al.* (2016) [29]
26.	Multi-class method for pesticides quantification in honey by means of modified QuEChERS and UHPLC-MS/	Brazil	Tette *et al.* (2016)
27.	Efficiency of QuEChERS approach for determining 52 pesticide residues in honey and honey bees	Spain	Vernich *et al.* (2016)
28.	Multi-residue analysis of pesticide residues in crude pollens by UPLC-MS/MS	China	Tong *et al.* (2016)
29.	Concentrations of neonicotinoid insecticides in honey, pollen and honey bees	Canada	Codling *et al.* (2016) [20]
30.	Pesticide residues in honey samples	Tamil Nadu, India	Hemalatha *et al.* (2018) [27]
31.	Validation of a multiresidue method for the determination of pesticides in honey bees by GC	Europe	Martel (2018) [36]
32.	Pesticide residues in Indian raw honeys	India	Kumar *et al.* (2018) [34]
33.	Pesticide residues in honey	Ghana	Darko *et al.* (2017) [21]
34.	Organochlorine pesticides in honey and pollen samples from managed colonies of *Apis mellifera*	Mexico.	Ruiz-Toledo *et al.* (2018)
35.	Pesticide residues in honey from stingless bee *Melipona subnitida*	Brazil	Corolina *et al.* (2020)
36.	Pesticide detection in honey using LGC coupled with MSD	Brazil	Almeida *et al.* (2020) [3]
37.	Pesticide residues in honey from stingless bee *Melipona subnitida*	Brazil	Pinheiro *et al.* (2020)
38.	Pesticide residues in Spanish honey measured by QuEChERS Method Followed by LGC-TMS	Spain	Roberto *et al.* (2021)
39.	Pesticides residues and metabolites in honeybees	Greek	Kasiotis *et al.* (2021) [31]
41.	Organochlorine pesticide residues in Uganda's honey.	Uganda	Mukiibi *et al.* (2021)
42.	Pesticide residues in Indian honey samples by LC-MS/MS and GC-MS/MS	India	Prasanth *et al.* (2022)
43.	Stingless bees: A review of the current threats to their survival	Mexico	Hernandez *et al.* (2022) [28]
44.	Multi-pesticide residues in honey with a modified QuEChERS procedure followed by LCMS/MS and GC-MS/MS	Turkey	Oymen *et al.* (2022)
45.	Honeybee exposure to multiple pesticide residues in the hive environment	China	Xiao *et al.* (2022)
46.	Pesticides in apicultural products	Spain	Murcia-Morales *et al.* (2022)
47.	Pesticide residue analysis in hive products of *Apis cerana indica*	Tamil Nadu, India	Yogapriya *et al.*, 2023

Table 2. Analysis of pesticide residues in stingless bee honey samples collected from different districts of southern Karnataka

Sl. No.	Pesticide	Stingless bee honey samples from		
		M	C	K
1.	Organochlorine: Aldrin, Chlordane, Chlormequat chloride, Dicofol, Endosulfan and Heptachlor	BDL	BDL	BDL
2.	Organophoshate: Acephate, Chlorpyrifos, Dichlorvos, Dimethoate, Edifenphos, Ethyl Parathion, Fenitrothion, Fenthion, Formothion, Malathion, Monocrotophos, Phosalone, Phosphamidon, Pirimiphos-methyl, Profenofos and Trichlorfon	BDL	BDL	BDL
3.	Organothiophosphate: Iprobenfos, Oxydemeton-methyl, Phenthoate, Quinalphos and Thiometon	BDL	BDL	BDL
4.	Organo Phosphorus: Fosetyl Aluminium and Phorate	BDL	BDL	BDL
5.	Organofluorine: Flubendiamide	BDL	BDL	BDL
6.	Pyrethroid: Alpha-cypermethrin, Beta-cyfluthrin, Etofenprox, Fluvalinate and Permethrin	BDL	BDL	BDL
7.	Herbicides: 2,4-Dichlorophenoxyacetic acid, 2,4-D Amine Salt, Alachlor, Ametryn, Anilophos, Atrazine, Azimsulfuron, Bensulfuron-methyl, Bentazone, Bispyribac sodium, Butachlor, Carfentrazone-ethyl, Chlorimuron-ethyl, Chlorpropham, Cinmethylin, Clodinafop-propargyl, Clomazone, Cyhalofop-butyl, Diclofop-Methyl, Diclosulam, Diuron, Ethoxysulfuron, Fenoxaprop-p-ethyl, Fluazifop-P-butyl, Flucetosulfurone, Fluchloralin, Fomesafen, Glufosinate-ammonium, Glyphosate, Halosulfuron-methyl, Haloxyfop-methyl, Hexazinone, Imazamox, Imazethapyr, Iodosulfuron-methyl-sodium, Isoproturon, Linuron, Mesosulfuron-methyl, Methabenzthiazuron, Methyl chlorophenoxy acetic acid, Metolachlor, Metribuzin, Metsulfuron-methyl, Orthosulfamuron, Oxadiargyl, Oxadiazon, Oxyfluorfen, Paraquat dichloride, Pendimethalin, Penoxsulam, Pinoxaden, Propanil, Propaquizafop, Pyrazosulfuron-ethyl, Pyrithiobac-sodium, Quizalofop-ethyl, Quizalofop-P-tefuryl, Simazine, Sodium acifluorfen, Sulfentrazone, Sulfosulfuron, Tembotrione, Topramezone, Triallate, Triasulfuron and Trifluralin	BDL	BDL	BDL
8.	Fungicides: Ametoctradin, Azoxystrobin, Benomyl, Bitertanol, Boscalid, Captafol, Captan, Carbendazim, Carpropamid, Chlorothalonil, Cyazofamid, Cymoxanil, Difenoconazole, Dimethomorph, Dithianon, Dithiocarbamates, Dodine, Epoxiconazole, Famoxadone, Fenamidone, Fenarimol, Ferbam, Fluopicolide, Fluopyram, Flusilazole (Organosilicon), Fluxapyroxad (Pyrazole-carboxamide), Hexaconazole, Iprodione, Isoprothiolane, Kresoxim-methyl, Mandipropamid, Metalaxyl, Metalaxyl-M, Metrafenone, Myclobutanil, Penconazole, Pencycuron, Picoxystrobin, Propiconazole, Propineb, Pyraclostrobin, Tebuconazole, Tetraconazole, Thifluzamide, Thiophanate-methyl, Triadimefon, Tricyclazole, Tridemorph, Trifloxystrobin and Validamycin	BDL	BDL	BDL
9.	Acaricide: Dinocap, Etoxazole, Fenazaquin and Milbemectin, Cyflumetofen, Fenpyroximate, Propargite and Hexythiazox.	BDL	BDL	BDL
10.	Insecticides: Abamectin, Aldicarb, Benfuracarb, Bifenthrin, Buprofezin, Carbaryl, Cartap hydrochloride, Chlorantraniliprole, Chlorfenapyr, Chlorfluazuron, Chromafenozide, Cyantraniliprol, DDT, Deltamethrin, Diafenthiuron, Dieldrin, Diflubenzuron, Dinotefuran, Emamectin benzoate, Ethion, Fenpropathrin, Fenvalerate, Fipronil, Flonicamid, Flupyradifurone, Imidacloprid, Indoxacarb (Oxadiazine pesticide), Lambda-cyhalothrin, Lindane, Lufenuron, Metaflumizone, Methyl parathion, Pymetrozine, Pyridalyl, Spinetoram, Spinosad, Spiromesifen, Spirotetramat, Sulfoxaflor, Thiocyclam Hydrogen Oxalate, Thiodicarb, Tolfenpyrad and Triazophos	BDL	BDL	BDL
11.	Pyrethroide: Cypermethrin; Plant Growth Retardant: Paclobutrazol	BDL	BDL	BDL
12.	Carbamate: Carbofuran, Carbosulfan, Methomyl, Fenobucarb	BDL	BDL	BDL
13.	Neonicotinoid: Acetamiprid, Thiacloprid, Thiamethoxam and Clothianidin	BDL	BDL	BDL
14.	Insect Growth Regulator: Pyriproxyfen	BDL	BDL	BDL
15.	Others: Antibiotic Compound: Kasugamycin; Plant Growth Regulators: Alpha naphthyl acetic acid, Ethephon, Forchlorfenuron, Hydrogen Cyanamide, Prohexadione calcium, Sodium para nitro phenolate, Triacontanol and Mepiquat chloride	BDL	BDL	BDL

Conclusion
Stingless bee honey samples collected from Mysore, Chamarajanagar and Kodagu districts are free from the heavy concentration of pesticides residues and they were present below detection limits and safe for human use as per the international standards.

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